

At page 3, paragraph 3, please delete this paragraph and substitute the following paragraph:

~~Preferably, the invention more particularly provides a peptide sequence comprising a region involved in interactions with sulphated glycoconjugates and in heparin binding. This peptide sequence is as follows:~~

KKAAPAKKAAPAKKAAPAKKAAAKKAPAKKAAAKKVTQK (SEQ ID No. 1)

At page 5, second paragraph, please delete this paragraph and substitute the following paragraph:

~~The present invention will now be described with reference to the following~~
Figures:

- Figure 1A is a graph illustrating an adhesion test carried out with CHO in the presence of increasing concentrations of D(+)galactose (black circles) or heparin (white circles) from pork intestinal mucosa.
- Figure 1B shows the effect of sulphated and non sulphated glucides on mycobacterial adhesion to CHO cells and to macrophages;
- Figure 2 shows data demonstrating purification of a heparin binding protein of *M. bovis* BCG;
- Figure 3 shows a comparison of the heparin binding protein of *M. bovis* with the antigen 85 complex;
- Figure 4 shows the effect of sulphated glucides and non sulphated glucides on haemagglutination induced by HBHA;
- Figure 5A is a graph illustrating the inhibition of adhesion of BCG to CHO cells by anti-HBHA monoclonal antibodies 3921E4;
- Figure 5B is a graph illustrating the inhibition of adhesion of BCG to CHO cells by anti-HBHA polyclonal antiserum;
- Figure 6 shows immunoblot analyses carried out with tuberculous anti-sera;
- Figure 7 shows the nucleotide sequence (SEQ ID No. 17) and the amino acid sequence (SEQ ID Nos. 17 and 18) of a fragment of HBHA deduced from a PCR fragment of chromosomal BCG DNA ;
- Figure 8 shows a Southern blot analysis of chromosomal BCG DNA;

- Figure 9 shows the sequencing strategy for the gene coding for HBHA;
- Figure 10 shows the DNA sequence of the BCG gene coding for HBHA (SEQ ID No. 19);
- Figure 11 shows a polyacrylamide gel electrophoresis and immunoblot analysis of the expression of HBHA in *E. coli*.

At page 6, paragraph 3, please delete this paragraph and substitute the following paragraph:

F4
The day before the adhesion test, the wells of 24-well tissue culture plates (Nunc, Nunc, Denmark) were inoculated with 10^5 extemporaneously cultivated cells from the ovaries of Chinese hamsters (CHO) (see Figure 1A, and Figure 1B, open bars) or J774A.1 macrophages (ATCC TIB67) (see Figure 1B, speckled bars) taken up into suspension in 2 ml of RPMI with an added 10% (v/v) of de complemented foetal calf serum (RPMI-FCS). Just before the test, the cells were washed three times with 2 ml of RPMI, and 1 ml of the mycobacterial suspension in RPMI was added to each well to obtain an infection multiplicity of 10 bacteria per eukaryote cell.

At page 9, fourth paragraph, please delete this paragraph and substitute the following paragraph:

FS
The capacity of bacterial adhesins to agglutinate erythrocytes is often used as a model to study microbial binding such as that of lectins to the receptor in eukaryotic cells. The inventors thus tested the purified heparin binding protein for its capacity to agglutinate erythrocytes.

At page 10, first full paragraph, please delete this paragraph and substitute the following paragraph:

F6
Less than 0.1 μ g of purified protein was able to induce haemagglutination of rabbit erythrocytes, but not of human, sheep, goose or chicken erythrocytes (data not shown). For this reason, the inventors designated this protein as being the heparin binding haemagglutinin (HBHA). Haemagglutination induced by HBHA was

inhibited by heparin or by dextran sulphate, but not by dextran (see Figure 4), which is similar to the results obtained in the tests for adhesion of mycobacteria to CHO cells. The haemagglutination activity was heat sensitive in that incubation of the protein for 60 minutes at 80°C destroyed haemagglutination.

At page 11, first full paragraph, please delete this paragraph and substitute the following paragraph:

F 7
The BCG was labelled with [6-³H]uracil as described above. Approximately 4 x 10⁶ radiolabelled BCG were taken up into suspension in 1 ml of PBS and pre-incubated with the indicated volumes of ascetic liquids of monoclonal antibodies 3921E4 (14) anti-HBHA (Figure 5A), or with 250 µl of rat anti-HBHA polyclonal antiserum (immunitary serum, Figure 5B) or naïve serum (Figure 5B, control serum) for 30 minutes, at room temperature. The hybridoma producing the 3921E4 anti-HBHA monoclonal antibodies was deposited on June 25, 2002, at the Collection Nationale de Cultures de Microorganismes (Institut Pasteur, 25 rue du Docteur Roux, Paris 15, France) under the registration number CNCM I-2900. The bacterial suspensions were then washed three times with 2 ml of DPBS to eliminate the non bound antibodies, then used in the CHO cell adhesion test in an infection multiplicity of 10, as described above.

At the last paragraph bridging pages 11 and 12, please delete that paragraph and substitute the following paragraph:

8
Purified heparin binding protein from a preparation of *M. bovis* BCG cell wall (Figure 3, track 1) or culture supernatant (track 2) was compared with purified antigen 85 complex (track 3) by immunoblot analysis (panels A, B, C) and staining with Coomassie Blue (panel D) after SDS-PAGE. Immunoblot analysis was carried out using polyclonal antibodies directed against the purified heparin binding protein (panel A), the monoclonal antibody 4057D2 (panel B) or polyclonal antibodies directed against antigen complex 85 (panel C). The hybridoma producing the 4057D2 anti-HBHA monoclonal antibody was deposited on June 25, 2002, at the Collection Nationale de Cultures de Microorganismes (see above) under the registration number CNCM I-2901. Tracks 1 and 2 of panels A, B and C contained 2

µg of purified protein, tracks 3 of panels A, B and C contained 7 µg of purified protein, and tracks 2 and 3 of panel D contained 4 µg and 15 µg of purified protein respectively. The size of labels M_r is shown in the margin.--

At page 12, second paragraph, please delete this paragraph and substitute the following paragraph:

F9
--The N-terminal amino acids of the purified heparin binding proteins of H37Ra *M. tuberculosis* and of BCG were sequenced. This was accomplished by subjecting 25 µg of HBHA to polyacrylamide-SDS gel electrophoresis using a 15% polyacrylamide gel. After electrophoresis, the material was transferred to a PVDF membrane (ProBlott, ABI) by electroblotting. After staining with Coomassie blue, the band corresponding to HBHA was excised and underwent automatised Edman degradation. The first 16 amino acids were Ala-Glu-Asn-Ser-Asn-Ile-Asp-Asp-Ile-Lys-Ala-Pro-Leu-Leu-Ala-Ala (SEQ ID No. 20 from amino acid positions 2 to 17). The first 16 amino acids in the BCG heparin binding protein were also determined and proved to be identical to those of *M. tuberculosis*. A similarity search in protein databases showed that the heparin binding protein had not been identified before, and that it thus represents a novel mycobacterial protein. Further, the first 16 amino acids did not exhibit any significant sequence similarity with other known protein sequences.--

At the last paragraph bridging pages 12 and 13, please delete that paragraph and substitute the following paragraph:

F10
--To clone the gene coding for HBHA, firstly the N-terminal sequences of internal HBHA fragments were determined. To this end, purified HBHA underwent electrophoresis as described above. After electrophoresis, the protein was digested with trypsin inside the gel. The resulting peptides were then isolated by reverse phase HPLC, and then underwent Edman degradation. Four peptides enabled the sequence to be determined:

Peptide	Lys-Ala-Glu-Gly-Tyr-Leu-Glu-Ala-Ala-Thr (SEQ ID No. 2)
S1441:	
Peptide	Xxx-Glu-Gly-Tyr-Val-Asp-Gln-Ala-Val-Glu-Leu-Thr-Gln-
S1443:	Glu-Ala-Leu-Gly-Lys (SEQ ID No. 3)
Peptide	Xxx-Gln-Glu-Xxx-Leu-Pro-Glu-Xxx-Leu (SEQ ID No. 4)
S1446:	
Peptide	Phe-Thr-Ala-Glu-Glu-Leu-Arg (SEQ ID No. 5)
S1447:	--

At page 13, first full paragraph, please delete this paragraph and substitute the following paragraph:

F¹¹ --The sequence of two pairs of oligonucleotides was derived from the internal HBHA peptide sequences. The generally high G+C content in the mycobacterial DNA has led the inventors to favour G or C in the third position of the codons (wobble). The first pair of oligonucleotides originated from the S1441 peptide and had the following sequences: 5'AAG GC(G/C) GAG GG(G/C) TAC CT 3' (oligo S1441) (SEQ ID No. 6) and 5' AGG TA (G/C) CCC TC(G/C) GCC TT 3' (reverse oligo S1441) (SEQ ID No. 7). The second pair of oligonucleotides originated from the S1443 peptide and had the following sequences: 5'GAC CAG GC(G/C) GT (G/C) GAG CT 3' (oligo S1443) (SEQ ID No. 8) and 5' AGC TC (G/C) AC(G/C) GCC TGG TC 3' (reverse oligo S1443) (SEQ ID No. 9) --

At page 13, second paragraph, please delete this paragraph and substitute the following paragraph:

F¹² --The chromosomal BCG DNA was extracted as described by Kremer et al (16). Polymerisation chain reactions (PCR) using 50 ng of chromosomal BCG DNA and 1 µg of either reverse S1441 and S1443, or reverse oligo S1443 and S1441, were carried out at a hybridization temperature of 50°C with 30 PCR cycles. Only the PCR carried out with the reverse S1441 and S1443 oligonucleotides produced a specific amplified DNA fragment of approximately 150 bp. This amplified fragment was again observed when the hybridisation temperature was increased to 57°C. --

At page 16, in Table I, please delete this Table and substitute the following Table:

TABLE I: Oligonucleotides used for sequencing the gene coding for HBHA.

Name of oligonucleotide	Sequence
HBHA Seq1	5'AGC CGG TAC AAC GAG CTG GTC 3' (SEQ ID No. 10)
HBHA Seq1inv	5'GAC CAG CTC GTT GTA CCG GCT 3' (SEQ ID No. 11)
HBHA Seq2	5'CAT CCA ACA CGT CGA CTC C 3' (SEQ ID No. 12)
HBHA Seq3	5'TTG ATG TCA TCA ATG TTC G 3' (SEQ ID No. 13)
HBHA Seq4	5'CGT GGA CCA GGC GGT GGA G 3'(SEQ ID No. 14)
HBHA Seq5	5'GAC GAT CAG GAG GTT TCC CCG 3'(SEQ ID No.15)
Reverse primer	5'AGC GGA TAA CAA TTT CAC ACA GGA 3' (SEQ ID No. 16)

At page 19, first full paragraph, please delete this paragraph and substitute the following paragraph:

The invention thus also concerns a recombinant peptide sequence characterized in that it enables mycobacteria to adhere to host cells. More particularly, the invention concerns a peptide sequence comprising a polypeptide of about 27 kDa recognized by the monoclonal antibody 3921E4 (14) and not recognized by the monoclonal antibody 4057D2 (14). Preferably, the recombinant peptide sequence of the invention is the expression product of a strain of *E. coli* transformed with one of the nucleotide sequences described above and coding for a peptide sequence enabling mycobacteria to adhere to host cells, more particularly a nucleotide sequence obtained from *M. bovis* BCG or *M. tuberculosis*. The invention also concerns any variation in this recombinant peptide sequence obtained by addition, substitution or deletion of one or more nucleotides such that the transformed strain produces a different peptide sequence but which possesses the property of adhering to host cells, and more particularly to epithelial cells.